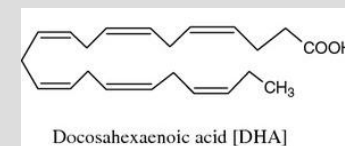
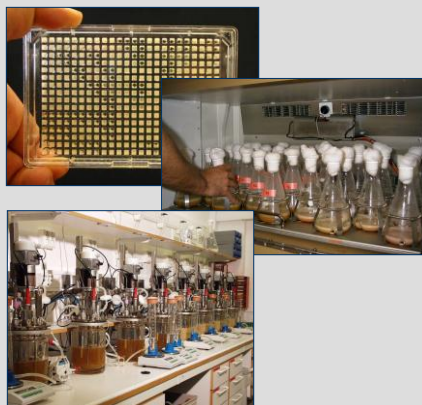
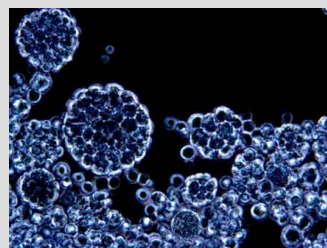


PRODUCTION OF DHA BY HETEROTROPHIC MICROORGANISMS

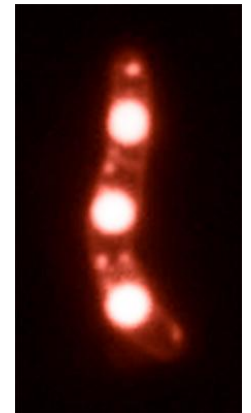
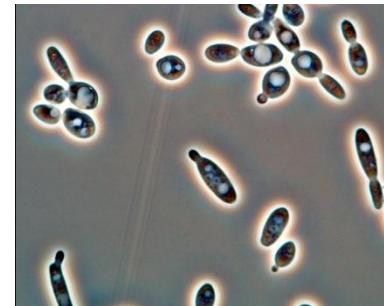
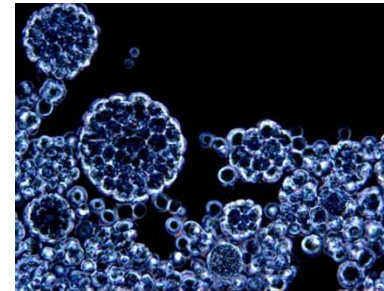


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SINTEF Materials and Chemistry

Heterotrophic ω 3-PUFA producing organisms

■ Organisms

- Lipid accumulating:
 - Microalgae
 - Thraustochytrids
 - Fungi and yeast
 - Bacteria
- Lipid accumulating and EPA/DHA producers:
 - Marine microalgae and thraustochytrids
 - Genetically engineered yeast



■ Carbon sources

- Typically glucose or sucrose

Physiology of lipid and EPA/DHA-production

- **Location of EPA/DHA in significant amounts:**
 - Membranes (phospho- and glycolipids):
 - Many marine, heterotrophic species, but membrane lipids <10 % of dw
 - Photosynthetic algae (chloroplast membranes)
 - Storage lipids (triacylglycerols):
 - Only *Cryptocodinium cohnii* and species of thraustochytrids
 - Accumulation is induced by nutrient limitation and energy in excess

Location of EPA / DHA (high levels, e.g. >20 % of TFA)	Organisms	Lipid content [% of dw]
Membrane lipids (glyco- and phospholipids)	Marine bacteria	<10
	Thraustochytrids	<10
	Heterotrophic microalgae	?
	Phototrophic microalgae	15 - ?
Storage lipids (triacylglycerol)	Thraustochytrids	> 50 (40-70)
	Heterotrophic microalgae	>50

Productivities

Heterotrophic organisms	Cell density [g dw/l]	DHA			Reference
		[% of dw]	[g/l]	[g/l·d]	
<i>C. cohnii</i>	109	17	19	1.2	De Swaaf et al., 2003
thraustochytrid strain 12B	21	27	5.6	2.8	Perveen et al., 2006
<i>S. limacinum</i> SR21	59	26	15.5	3.0	Yaguchi et al., 1997
<i>S. limacinum</i> SR21	62	32	20	2.9	Huang et al., 2012
<i>Aurantiochytrium</i> sp.	90-100	15	14	2.2	Jakobsen et al., 2008
<i>Schizochytrium</i> sp.	160-180	25	40-45	10-12	US 7732170

Phototropic algae	1-4	3-5	0.05-0.2	≤ 0.06
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Production technology

■ Fermentation

- Well-established large scale technology, with an annual production of:
 - 2.5-3 mill. tonnes amino acids (lysine and glutamate)
 - 1.5-2 mill. tonnes citric acid
 - Production plants comprising >10 reactors of 300-500 m³



Required capacities for feed applications

25 000 tonne EPA/DHA (100 000 tonnes "fish oil equivalents")

Productivity [g/l-d]	Reactor volume [m ³]	Plant size	Technology status	Ground area
10	6 850	15-20 reactors á 350-450 m ³	Existing	~1000 m ²
0.06	1 141 550	Pond: 6300 ha*	Need to be developed	20-60 km ²
		Tubular: 2550 ha*		
		Flat panel: 1980 ha*		

*: Ground area; ratios (m³/ha) from Norsker et al. (2011)

Costs

- Calculations based on cost analyses and information on large scale fermentation processes for amino acids
- A "value production" of 125-250 €/m³,day is required for an economical feasible process (15 % internal rate of return)

Required selling price for DHA as a function of productivity for 150 €/m³,d "value production"

Productivity [g/l·d]	Selling price [€/kg]			
	DHA	Oil		
		40 %*	25 %*	10 %*
5	30	12.00	7.50	3.00
10	15	6.00	3.75	1.50
15	10	4.00	2.50	1.00
25	6	2.40	1.50	0.60

*: % DHA of TFA

Fish oil: ~ 1 €/kg, corresponding to 4 €/kg EPA/DHA (or 10 €/kg DHA)

Phototrophic: 3 €/kg dw (Norsker et al., 2011).

If 5 % EPA-> Production costs 60 €/kg, selling price (+50 %) 90 €/kg

Optimization and cost reduction

Potential improvements

- Increased product concentration
 - Cell density
 - Lipid content
 - DHA content of TFA
- Increased growth and production rates

	Cell density [g/l]	TFA [% of dw]	DHA [% of TFA]	Ferm time [h]	DHA productivity [g/l·d]
Current reported	160-180	60-65	40-45	80-90	10-12
Maximum reported for each parameter	180	65	67	80	24
Assumed max for each parameter	200	70	70	70	34

Sustainability

■ Carbon sources

- A production of 100 000 t "fish oil equivalents" (25 000 t EPA/DHA) will represent ~2 % of the current global fermentation production based on glucose (starch) and sucrose.
- Wastes, such as glycerol, can be utilized
- Lignocellulose and seaweed biorefineries can represent a future carbon source

■ Other issues that have to be considered

- Energy
- Water
- Other nutrients
- etc.

Conclusions

■ Heterotrophic production

- Technology well established
- Costs
 - Maximum reported productivities correspond to selling prices of 15 €/kg DHA (several reports -> ~30 €/kg)
 - Process optimization (reduce time, increase lipid and DHA-fraction) => <10 €/kg DHA
- In a foreseeable future:
Probably the only realistic technology for production of the required amounts to supply the aquaculture industry

But:

- A systematic comparison of the technologies on the same basis (e.g. production volumes) should be carried out:
 - Costs
 - Need for genetic engineering to improve productivity; realistic achievements with and without
 - For phototropic production:
 - Technology development (increased photosynthetic efficiency)
 - Location
 - Etc.
 - Sustainability (carbon sources, other nutrients, energy, water, location etc.)
 - Product properties (e.g. polar lipids vs triacylglycerol), other valuable compounds (e.g. pigments) etc.

... and, phototropic production may be more competitive for smaller volumes (and other products)!?